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# Somitogenesis: Breaking New Boundaries

Minireview

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**Segmentation is a fundamental process in vertebrate embryogenesis, and one of the earliest manifestations of segmental patterning is the generation of transient, serially repeated blocks of mesodermal cells known as somites. Disruption of the normal segmentation process in humans leads to vertebral abnormalities such as spondylocostal dysostosis. In this minireview, we discuss recent advances in the dynamic molecular and cellular mechanisms governing segmentation.**

### *The Somitic Clock: Notching up Segments in a Lunatic World*

In contrast to the chaotic pace of our lives, the vertebrate embryo is patterned in a far more controlled fashion, with a somite being formed every 20 min in zebrafish, 90 min in chick, and 2 hr in mice. The number of somites generated in vertebrates varies from as few as 42 in humans to around 65 in mice to as many as nearly 400 in some species of snakes. Somites provide the blueprint for the construction of the vertebral column and muscle groups at different axial levels of the body. Somites also impart a periodic restriction in the position of the ventral spinal roots of the spinal cord, influence the migration of neural crest cells, and determine the placement of the spinal ganglia. The remarkable consistency in both the number and morphology of the segmental units produced during development within a species suggests that the act of segmentation is subject to stringent regulation. Several mechanisms including a "clock and wavefront" model, a "clock and trail" model, and a "cell cycle" model have been hypothesized in order to account for this periodicity in the segmentation program (Cooke and Zeeman, 1976; Kerszberg and Wolpert, 2000; Stern et al., 1988). Integral to each of these models is an oscillator that drives segmentation. The first molecular evidence for the existence of a segmentation clock came from the discovery of oscillating *c-hairy1* expression in the presomitic mesoderm (psm) of avian embryos (Palmeirim et al., 1997). The posterior-to-anterior wave-like propagation of gene expression is not caused by cell movement but is the result of the synchronous oscillation in the psm. Since then, several other genes in different species with dynamic synchronous cycling expression have also been identified. These include *c-hairy2*, *lunatic fringe* (*Lfng*), *deltaC*, *her1*, *her7*, *Hes1, Hes7*, and *Hey2,* all of which are involved in *Notch* signaling, suggesting that *Notch* lies at the heart of the oscillator. Each of these genes displays the same dynamic pattern that can be divided into three phases (Figure 1). Phase I involves a broad expression domain throughout the caudal psm. In phase II, the domain shifts to the middle of the psm. This posterior-to-anterior sweep of expression refines in phase III to include a strong band across the presumptive somite border in the rostral-most compartment of the psm, which is often accompanied by weaker expression in the caudal psm. At the end of phase III, the single oscillation of the somitogenesis cycle is accompanied by the emergence of a new somite (Pourquie and Tam, 2001).

Genetic analyses have determined that these oscillating genes play critical roles in somitogenesis since mutations in *deltaC, her1*, and *her7* in zebrafish (Henry et al., 2002; Oates and Ho, 2002) and *Lnfg* and *Hes7* in mouse (Bessho et al., 2001; Evrard et al., 1998; Zhang and Gridley, 1998) all exhibit segmentation defects. In zebrafish, oscillating *deltaC* has been hypothesized as the driver of periodic *Notch* activation, which is ultimately responsible for the cyclic expression of the b-HLH (basic helix loop helix) repressors *her1* and *her7* (Jiang et al., 2000; Oates and Ho, 2002). *her1* and *her7* then, in turn, regulate their own expression via a negative feedback loop. In contrast, a *Lfng*-mediated negative feedback loop inhibits *Notch* signaling in avians (Dale et al., 2003). The murine *Lnfg* promoter is regulated by a number of cis-regulatory "clock elements" that contain CBF1 binding sites and E boxes, suggesting that the cyclic genes are directly regulated by *Notch* and the b-HLH transcriptional repressors. In support of this idea, mutations in *Hes7* inhibit *Lfng* oscillations, demonstrating that *Hes7* plays a critical role in regulating the segmental clock in mice. Furthermore, periodic repression by HES7 protein is critical for the cyclic transcription of both *Hes7* and *Lfng*, indicating that negative feedback loop regulation of *Notch* signaling could provide the molecular foundations of the segmentation clock (Bessho et al., 2003), similar to negative feedback loops in other species.

*Notch* activation clearly plays a central role in the oscillator of all vertebrate species analyzed to date, and in mutant mouse embryos, disruption of the Notch signaling pathway prevents the oscillations of the cyclic genes (Jouve et al., 2000). Surprisingly, boundaries can still form in *Notch* null mutant mice (Conlon et al., 1995), and RNAi disruption of *Notch* signaling in spiders disorganizes rather than abolishes segmentation (Stollewerk et al., 2003). Hence, although the precise role for *Notch*-dependent periodic gene expression in the segmentation process remains unresolved, recent work in spiders suggests that *Notch* signaling may be an evolutionary conserved integral component of ancestral segmentation (Stollewerk et al., 2003). These analyses, however, raise the possibility of other oscillating molecular pathways being involved in the segmentation process.

Indeed, a second distinct group of cyclic genes has now been characterized, which plays an integral role in the segmentation clock via the Wnt signaling pathway (Aulehla et al., 2003). *Axin2*, a negative regulator of Wnt signaling, is expressed dynamically within the presomitic mesoderm, phase shifted with respect to *Lfng* periodicity (Figure 1). Ectopic expression of *axin2* in mouse presomitic mesoderm leads to an upregulation of *Lfng*

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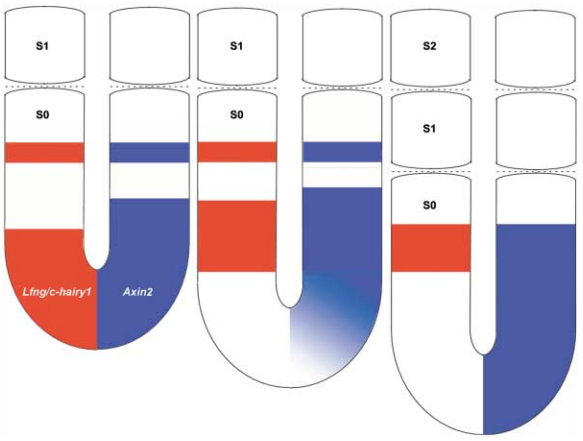


Figure 1. Oscillation of *Lfng/c-hairy1* and *Axin* during Somitogenesis

Comparison of the three phases of *Lfng/c-hairy1* (red) and *Axin2* (blue) expression during each oscillation of the somitogenic clock. Note that *Axin2* expression is out of phase with *Lfng/c-hairy1*. S0 is the next somite to form. S1 is the most recently formed somite. S2 is the somite formed prior to S1.

and the loss of the proper phased expression of the gene (Aulehla et al., 2003). Conversely, *axin2* levels are downregulated in the presomitic mesoderm of hypomorphic *Wnt3a* mutants, which nonetheless maintains *Notch1* and *Dll1* expression. *Axin2* therefore appears to be a direct transcriptional target of Wnt signaling, acting downstream of *Wnt3a*. In support of this, the *axin2* promoter contains functional *TCF/LEF* binding sites, the transcriptional effectors of Wnt signaling (Aulehla et al., 2003). One explanation for these observations is that *axin2* integrates Wnt signaling into the Notch-driven somitogenesis clock and that this signal is regulated through a negative feedback loop. Wnt signaling therefore potentially plays a primary functional role in the gradient and oscillation process that regulates segmentation.

The segmentation clock appears to control some aspects of spatiotemporal *Hox* gene activation (Dubrulle et al., 2001; Zakany et al., 2001), thereby ensuring a correlation between segment boundary position and future regional identity of the somites. What is the basis for this mechanism? FGF, Wnt, and retinoic acid (RA) signaling all have a demonstrated ability to influence rostrocaudal patterning, and interestingly, *Wnt3a* and *Fgf8* exhibit overlapping expression domains in the presomitic mesoderm. Wnts and FGFs act synergistically to pattern the neural plate with increasing concentrations of Wnt activity, inducing more caudal neural tissue, and it is possible that a similar partnership could also be involved in regulating the somitogenic oscillator. Currently, *Wnt3a* appears to function upstream of *Fgf8*. Altering the dynamics of the FGF8 gradient affects the position of somite boundaries (Dubrulle et al., 2001), while *Wnt3a* mutants exhibit axial truncations posterior to the level of the hindlimbs (Greco et al., 1996). Does this imply that there are two oscillation phases or organizing regions in which anterior somites are regulated by *Fgf8* (Dubrulle et al., 2001) while those posterior to the hindlimbs are governed by Wnt signaling? Recent work in zebrafish has identified a tail bud organizer that functions through the combined interactions of BMP, nodal, and Wnt8 signaling, which is clearly distinct from the head organizer (Agathon et al., 2003). It is also worth remembering that RA, a potent posteriorizing morphogen and activator of *Hox* genes, inhibits *Wnt3a* in the posterior presomitic mesoderm, resulting in tail bud agenesis and a transformation of mesoderm to a neuroectodermal fate (Iulianella et al., 1999; Shum et al., 1999). It is not known if the segmentation clock was suppressed in these experiments; however, the involvement of RA adds another level of complexity to the mechanism that functionally integrates the oscillating clock and the acquisition of anterior-posterior patterning into the generation of segment boundaries.

***Somite Formation: The Ball and Socket Mechanism***

Somites form through the epithelialization of groups of cells in the paraxial mesoderm concomitantly with their separation from the anterior end of the unsegmented paraxial mesoderm. This is accomplished by changes in cellular behavior and tissue architecture such that balls of cells in the paraxial mesoderm become separated in a craniocaudal succession along the body axis. Concomitantly, borders between adjacent groups of cells (the space that constitutes the intersomitic fissures) are generated in a spatially and temporally precise manner. Recently, exciting new in vitro time-lapse imaging has detailed the precise cell movements that occur during somite formation with some surprising results (Kulesa and Fraser, 2002).

Somite formation commences with the recruitment of mesenchymal cells into the paraxial mesoderm from a progenitor population found initially in the primitive streak during gastrulation and later in the tail bud. As these cells enter the posterior end of the segmental plate, they disperse widely. However, their movements become progressively more restricted the closer these cells come to the anterior of the segmental plate prior to somite segmentation. Somite segmentation is not a simple slicing process as previously thought, but rather a more complex process that involves the choreographed movements of cells across presumptive somite boundaries (Figure 2; Kulesa and Fraser, 2002). Initially, a tongue of cells adjacent to the neural tube at the anterior-lateral-most end of the presomitic mesoderm separates from the segmental plate, creating a wedge of cells divided by a small cleft. Cells in the medial part of the segmental plate, posterior to the presumptive boundary, coalesce to form a cluster. Subsequently, the lateral tongue and medial cell cluster slide posteriorly and anteriorly, respectively, across the presumptive somite boundary, reversing their original axial relationship. While the medial cell cluster is absorbed into the forming somite (S1) and contributes to its posterior border, the anterior-lateral tongue of cells folds medially, becoming part of the anterior border of the next forming somite (S0). These extraordinary cell movements resemble a ball and socket, and intriguingly, these cell movements are associated with rapid differential gene expression changes.

The receptor tyrosine kinase *EphA4* is a bidirectional signaling molecule that mediates repulsive interactions between cells and was previously shown to play an important role in somite segmentation (Xu et al., 1999). Interestingly, the lateral tongue of cells that initiates S0 separation does not express *EphA4*. However, as this tongue of cells folds medially, it merges with a domain of high *EphA4* expression, and consequently these cells begin to express *EphA4* themselves. Conversely, the medial cell cluster arises in an area of high *EphA4* expression but moves to an area of low expression and concomitantly downregulates

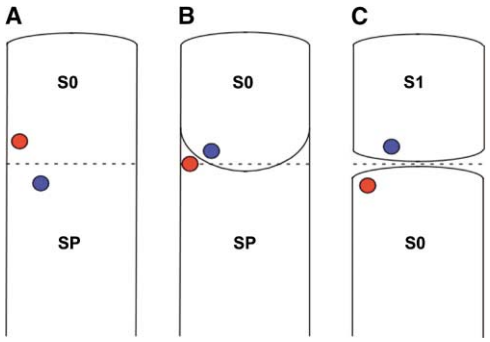


Figure 2. Somite Segmentation Mechanism

Somite segmentation is not a simple slicing process, but involves dynamic ball and socket cell movements across presumptive boundaries.

(A) Initially a tongue of cells in the anterior-lateral-most end of the presomitic mesoderm (red cell) separates from the segmental plate (SP), creating a wedge of cells divided by a small cleft. Cells in the medial part of the segmental plate (blue cell) are posterior to the presumptive boundary.

(B) Subsequently, the lateral tongue and medial cells slide posteriorly and anteriorly, respectively, across the presumptive somite boundary, reversing their original axial relationship.

(C) While the medial cells are absorbed into the forming somite (S1) and contribute to its posterior border, the anterior-lateral tongue of cells becomes part of the anterior border of the next forming somite (S0).

*EphA4* (Kulesa and Fraser, 2002). This refinement of gene expression at newly formed borders is similar to that seen during the formation of hindbrain rhombomeres, where bidirectional repulsion, cell plasticity, and response to community effects serve to progressively sharpen individual boundaries (Trainor and Krumlauf, 2000). In contrast to the hindbrain, where *ephrins* and *Eph* receptors initiate cell sorting and compartment formation, in the segmental plate, *Eph* receptors and their *ephrin* ligands do not appear to be responsible for initiating the somite compartment boundary, but may play an important role in maintaining it. However, this assumption is made on the basis of RNA gene expression and still needs to be confirmed at the protein level.

These ball and socket cell movements are tightly choreographed and coordinated with the final epithelialization step in the somite segmentation process. Epithelialization of the forming somite (S0) begins at its anterior-medial edge and proceeds radially, in an anterior-to-posterior direction, finishing at its posterior edge as it separates from the segmental plate to become S1 (Kulesa and Fraser, 2002). The newly formed somite exists as a core of mesenchymal cells surrounded by a ring of epithelial cells. Similar analyses have also noted that an epithelium forms along the lateral side of the somite prior to somite separation, leading to the hypothesis that it is distinct from the formation of the epithelium at the somite boundary (Rhee et al., 2003). The differential regulation of boundary versus lateral epithelium appears to be supported by the fact that embryos lacking *Lfng*, *Mesp2*, and *Papc* all demonstrate a disruption in epithelialization at the somite boundary only, while the lateral epithelium remains intact (Rhee et al., 2003). The idea that the boundary epithelium is differentially regulated from the lateral epithelium is an intriguing phenomenon, and the question that remains is how genes that play a role in the formation of somites—the oscillating genes of the Notch pathway, along with *Eph* receptors and their *ephrin* ligands, FGFs and Wnts—correlate with such complex cell movements, especially when cell movement occurs across presumptive somite boundaries. One possibility is that the cyclic genes may regulate cell segregation and somite separation by instituting changes in cell adhesion as well as cell attraction and repulsion. In support of this idea, differences in the repertoire of adhesion molecules expressed by cells at a boundary can lead to cell sorting. A recent study demonstrates that the protocadherin *Papc*, which is expressed in the anterior regions of S0 and S1, exhibits a cyclical pattern of expression (Rhee et al., 2003). *Papc,* which is necessary for appropriate epithelialization of the somite boundary, is regulated by *Lfng*. Furthermore, generating discrete ectopic domains of *Lfng* activity in the unsegmented paraxial mesoderm induces fissures to form at the interface (Sato et al., 2002) and may therefore represent a true connection between the segmentation clock and morphologic events associated with segmentation. A future challenge will be to determine what genetically initiates the epithelialization process in such a site-specific and spatiotemporal manner.

### *The Syndetome: A Somitic Compartment for Tendon Precursors*

The development of the skeleton and skeletal muscle from somites has been well characterized. It has long been known that once the epithelialized somite has formed, it responds to signals from surrounding tissues and divides into two initial compartments, the sclerotome and dermomyotome. The mesenchymal sclerotome is formed when the ventromedial portion of the somite undergoes an epithelial-mesenchymal transition, separating from the dorsolateral dermomyotome. Later, the ventromedial and dorsolateral lips of the dermomyotome delaminate and migrate underneath the compartment to form the myotome. The sclerotome will go on to form the vertebrae and ribs, the myotome will form the skeletal muscle progenitors associated with the axial skeleton, and the dermotome will give rise to the dermis.

To create a functional musculoskeletal system, however, muscle must be tightly attached to bone—a role fulfilled by tendons. Although development of the skeleton and axial muscle is well understood, until recently our knowledge of tendon patterning was poor, due primarily to the lack of a molecular marker for this population. New work has now characterized a previously undescribed fourth somitic compartment, called the syndetome, from which the tendon progenitors arise (Figure 3; Brent et al., 2003). The syndetome is derived from a dorsolateral domain of the sclerotome, forming at the juxtaposition of the sclerotome and myotome, and can be identified by expression of the bHLH transcription factor *Scleraxis (Scx)*. *Scx* expression does not overlap with *Pax1* or *MyoD* domains of expression, indicating that the syndetome is a discrete and unique somitic compartment completely separate from the sclerotome and myotome. Cells expressing *Scx* appear to be directed to a unique fate contributing only to the formation of tendons. *Scx* is expressed in limb and axial tendons during later embryogenesis, which is indicative of the unique fate of cells derived from the syndetome (Brent et al., 2003).

The critical spatial positioning of the syndetome between the sclerotome and myotome raises the question of how the syndetome is induced. Ablation of the dermomyotome results in the loss of *Scx* expression and the concomitant absence of the syndetome. Subsequent bead implant and overexpression analyses have now de-

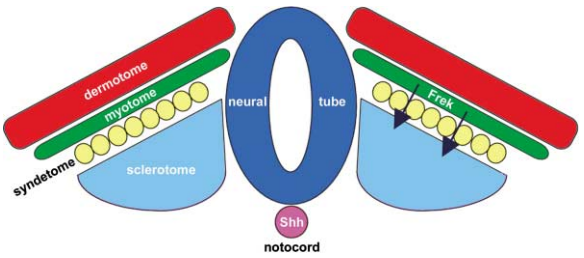


Figure 3. The Syndetome, a Somitic Home for the Tendons

The tendon precursors are found in a discrete somitic compartment termed the syndetome (yellow cells). The syndetome is derived from the sclerotome (blue) and is induced to form at the anterior and posterior medial edges of the somite at the juxtaposition of the sclerotome and myotome (green). FGF signaling from the center of the myotome is relayed by the *Frek* receptor that induces *Scx* and the syndetome. *Shh* expression from the notocord (purple) and/or floor plate represses syndetome formation.

termined that *Fgf8* signaling from the central region of the myotome is relayed by its receptor *Frek*, which is expressed in the anterior and posterior edges of the myotome, to induce *Scx* in a complimentary domain in the underlying sclerotome (Figure 3; Brent et al., 2003). Control of *Scx* expression and syndetome formation, however, is more complex than simple regulation by FGF signaling. *Pax1* is initially expressed throughout the sclerotome but subsequently becomes downregulated dorsomedially and ventrolaterally in a domain that corresponds to the future expression domain of *Scx*. Overexpressing *Pax1* or inducers of *Pax1* such as *Shh* inhibits *Scx* expression and hence syndetome formation. Interestingly, FGF8 downregulates *Pax1*, but this alone is insufficient to mediate *Scx* expression and is indicative of the complexity of syndetome induction. The current mechanism for syndetome formation is that FGF8 signaling mediated by its receptor *Frek* in the myotome controls the formation of the syndetome from sclerotomal cells via the simultaneous induction of *Scx* and repression of *Pax1* (Brent et al., 2003). The identification and characterization of the syndetome is an important advance in somitogenesis because it demonstrates how tight spatial and temporal regulation coordinates the complex development of the sclerotome, syndetome, myotome, and dermotome.

### *Future Perspectives*

Despite these rapid advancements in our understanding of the genetic and cellular mechanics of vertebrate somitogenesis, a number of challenges still remain. Of key importance is the control of somite number, which varies between species. Is the number of oscillations a consequence of intrinsic node or primitive streak stem cell population characteristics or some other mechanism? Furthermore, we still do not know what initiates and then subsequently stops the oscillator. FGF, Wnt, and RA signaling could all be integral to this process; however, it will be necessary to precisely account for the independent functions of these signaling pathways in the oscillator, similar to what has already been described in neural patterning. Comparative analyses between diverse species such as snakes and avians should be instrumental in uncovering the mechanisms that regulate the oscillator and somite number. Furthermore, these analyses will also be fundamental in deciphering how the oscillator and the generation of anterior-posterior positional information along the vertebrate axis are functionally integrated.

Another outstanding issue in somitogenesis is how the initial registration between somite compartments such as the sclerotome, syndetome, and myotome relates to skeletal, tendon, and muscle differentiation. Sophisticated time-lapse observation of the precise relative migration of cells from these discrete somite compartments is required to determine whether these cells maintain their spatial relationships during somite differentiation. These types of analyses are necessary to uncover the unique mechanisms that generate a fully and functionally integrated musculoskeletal system.

### Selected Reading

Agathon, A., Thisse, C., and Thisse, B. (2003). Nature *424*, 448–452.

Aulehla, A., Wehrle, C., Brand-Saberi, B., Kemler, R., Gossler, A., Kanzler, B., and Herrmann, B.G. (2003). Dev. Cell *4*, 395–406.

Bessho, Y., Sakata, R., Komatsu, S., Shiota, K., Yamada, S., and Kageyama, R. (2001). Genes Dev. *15*, 2642–2647.

Bessho, Y., Hirata, H., Masamizu, Y., and Kageyama, R. (2003). Genes Dev. *17*, 1451–1456.

Brent, A.E., Schweitzer, R., and Tabin, C.J. (2003). Cell *113*, 235–248.

Conlon, R.A., Reaume, A.G., and Rossant, J. (1995). Development *121*, 1533–1545.

Cooke, J., and Zeeman, E.C. (1976). J. Theor. Biol. *58*, 455–476.

Dale, J.K., Maroto, M., Dequeant, M.L., Malapert, P., McGrew, M., and Pourquie, O. (2003). Nature *421*, 275–278.

Dubrulle, J., McGrew, M.J., and Pourquie, O. (2001). Cell *106*, 219–232.

Evrard, Y.A., Lun, Y., Aulehla, A., Gan, L., and Johnson, R.L. (1998). Nature *394*, 377–381.

Greco, T.L., Takada, S., Newhouse, M.M., McMahon, J.A., McMahon, A.P., and Camper, S.A. (1996). Genes Dev. *10*, 313–324.

Henry, C.A., Urban, M.K., Dill, K.K., Merlie, J.P., Page, M.F., Kimmel, C.B., and Amacher, S.L. (2002). Development *129*, 3693–3704.

Iulianella, A., Beckett, B., Petkovich, M., and Lohnes, D. (1999). Dev. Biol. *205*, 33–48.

Jiang, Y.J., Aerne, B.L., Smithers, L., Haddon, C., Ish-Horowicz, D., and Lewis, J. (2000). Nature *408*, 475–479.

Jouve, C., Palmeirim, I., Henrique, D., Beckers, J., Gossler, A., Ish-Horowicz, D., and Pourquie, O. (2000). Development *127*, 1421–1429.

Kerszberg, M., and Wolpert, L. (2000). J. Theor. Biol. *205*, 505–510.

Kulesa, P.M., and Fraser, S.E. (2002). Science *298*, 991–995.

Oates, A.C., and Ho, R.K. (2002). Development *129*, 2929–2946.

Palmeirim, I., Henrique, D., Ish-Horowicz, D., and Pourquie, O. (1997). Cell *91*, 639–648.

Pourquie, O., and Tam, P.P. (2001). Dev. Cell *1*, 619–620.

Rhee, J., Takahashi, Y., Saga, Y., Wilson-Rawls, J., and Rawls, A. (2003). Dev. Biol. *254*, 248–261.

Sato, Y., Yasuda, K., and Takahashi, Y. (2002). Development *129*, 3633–3644.

Shum, A.S., Poon, L.L., Tang, W.W., Koide, T., Chan, B.W., Leung, Y.C., Shiroishi, T., and Copp, A.J. (1999). Mech. Dev. *84*, 17–30.

Stern, C., Fraser, S., Keynes, R., and Primmett, D. (1988). Development *104*, 231–244.

Stollewerk, A., Schoppmeier, M., and Damen, W.G. (2003). Nature *423*, 863–865.

Trainor, P., and Krumlauf, R. (2000). Nat. Rev. Neurosci. *1*, 116–124.

Xu, Q., Mellitzer, G., Robinson, V., and Wilkinson, D. (1999). Nature *399*, 267–271.

Zakany, J., Kmita, M., Alarcon, P., de la Pompa, J.L., and Duboule, D. (2001). Cell *106*, 207–217.

Zhang, N., and Gridley, T. (1998). Nature *394*, 374–377.